REMARKS

In this Amendment, claims 19-22 are added. After entry of this Amendment, which is respectfully requested, claims 3, 5 and 19-22 will be all of the claims pending in the Application.

New claims 19-22 are supported by the specification at page 10, first full paragraph.

The present invention, for example as recited in claim 3, is a method for detecting a protease in a biological sample comprising the steps of (1) contacting one of two or more substantially continuous slices of a biological sample with a dried thin membrane which comprises a protease substrate together with a cross-linking agent formed on a surface of a support; (2) contacting the remaining slices with a dried thin membrane which comprises a protease substrate, a cross-linking agent, and a protease inhibitor formed on a surface of a support; (3) detecting traces of digestion formed on the dried thin membranes by the action of protease; and (4) comparing the trace of digestion on the dried thin membrane used in step (1) with the trace of digestion on the dried thin membrane used in step (2).

I. Rejection Under 35 U.S.C. § 103(a)

(1) At page 3 of the Office Action, claim 3 is rejected under 35 U.S.C. § 103(a) as obvious over Salthouse *et al.* (Experientia, 1970) in view of Galis *et al.* (*FASEB*, 1995) and Battista (U.S. Patent 3,649,347). Specifically, the Examiner states that Salthouse teaches a method for detecting protease in a sample by contacting tissue sections with a dried thin

membrane comprising crosslinked collagen on a support, and comparing digestion of the collagen to digestion of collagen soaked in a protease inhibitor. The Examiner states that Galis teaches a method of detecting a protease in a biological sample by contacting consecutive tissue sections with a thin membrane. The Examiner also states that Battista teaches a variety of crosslinking agents for collagen and teaches that crosslinking may improve the properties of thin collagen films.

The Examiner concludes that one of ordinary skill in the art, wanting to assay for protease activity across a series of tissue sections, would have been motivated to perform the method of Salthouse using the consecutive tissue sections as taught by Galis or as suggested by salthouse. The Examiner also contends that one of ordinary skill in the art wanting to improve the properties of the collagen film of Salthouse would have been motivated to add the crosslinking agents to the collagen film as taught by Battista. The Examiner asserts that one of ordinary skill in the art would have had a reasonable expectation of successfully applying the teachings of Battista to the assay of Salthouse, because Battista teaches that crosslinked collagen may be used successfully on a variety of surfaces, and for a variety of purposes.

(2) At page 5 of the Office Action, claim 5 is rejected under 35 U.S.C. § 103(a) as obvious over Salthouse in view of Galis and Battista, as applied above, and further in view of Lawrence *et al.* (U.S. Patent 5,416,003).

Specifically, the Examiner states that Lawrence teaches a device for detecting proteases in samples with multiple layers. The Examiner states that one of skill in the art would have been motivated to laminate a layer comprising a substrate, a cross-linking agent, and an inhibitor, to a

layer comprising a substrate and cross-linking agent, in order to measure protease with the method of Salthouse in a single sample with a single test element. The Examiner concludes that one of skill in the art would have had a reasonable expectation of successfully applying the teachings of the cited prior art to perform the claimed method because Battista teaches that crosslinked collagen may be applied to a variety of supports and Lawrence teaches that multiply-layered elements may be used to detect proteases.

II. Response to Section 103(a) Rejections

Initially, Applicants respectfully submit that Salthouse does not teach or suggest crosslinked collagen as the Examiner states. Salthouse merely discloses a collagen dispersion, without any teaching or suggestion of adding a crosslinking agent. Therefore, Battista is the only cited art of record that discloses any crosslinking agent.

Applicants further submit that Battista does not motivate one skilled in the art to add cross-linking agents to the recited thin dried membrane for use with the claimed method of measuring protease in a biological sample, and as such, claims 3 and 5 are not obvious.

Battista states at column 3, line 12-15, that the crosslinking agents are added to "impart substantial wet strength to the films." Battista further states at column 6, lines 46-51, that crosslinking agents, in addition to improving the wet-strength, improve the heat-resistance of the collagen films.

Applicants submit that one of skill in the art would not have been motivated to measure protease in a biological sample, by contacting the sample with a dried thin membrane comprising

crosslinked collagen, based on Battista's disclosure because, while "wet-strength" may provide an advantage to the collagen sutures, mats, and papers of Battista, "wet-strength" is irrelevant to the thin dried membrane recited in the claims. When the thin dried membrane recited in claims 3 and 5 are used for measuring protease in a biological sample, the membrane is not rubbed or otherwise stressed such that "wet-strength" would be relevant to the claimed method.

Further, with regard to heat resistance, Battista states that when certain crosslinking agents are used, shrinking and discoloration of the film are less upon heating. See Battista at column 6, lines 46-51. For example, Battista states at column 8, lines 27-34, that collagen films containing the crosslinking agent melamine formaldehyde precondensate (see also col. 6, line 32) were dried at 250-275° C. Applicants submit that the presently claimed methods are preferably performed at 37° C (see specification at, for example, page 14, line 12; page 16, line 18; and page 17, line 11). Thus, while heat resistance may impart an advantage to the paper of battista, heat resistance is not relevant to performing the claimed method of measuring a protease in a biological sample using a dried thin membrane.

In addition, Applicants submit that crosslinking agents provide an advantage to the claimed method that is unexpected over the prior art of record. The crosslinking agent is added to the dried thin membrane to control the degree of protease substrate digestion by a protease. Thus, the crosslinking agent allows one to control the sensitivity of the method so as to be suitable for detection of a protease in a biological sample, and the amount of crosslinking agent added to the membrane is defined in the specification from this view of sensitivity.

For example, sample Nos. 116, 117, 118, and 127 (see Table 1, beginning at page 21 of the specification) were prepared so as to contain a different amount of the cross-linking agent 1,2-Bis (vinylsulfonyl-acetoamido) ethane. In is readily understood from the experimental results obtained with these samples that a higher amount of the crosslinking agent reduces the degree of digestion by a protease (see Table 5 at page 29). For example, the higher amount of crosslinking agent in sample 118 reduces protease digestion over the lesser amounts contained in samples 116 and 117.

Battista, which teaches adding crosslinking agents to collagen films to improve the wet-strength and/or heat resistance of sutures, mats, and paper, does not motivate one skilled in the art of measuring protease in biological samples to use a crosslinking agent for the purpose of controlling the sensitivity of a method of measuring such a protease.

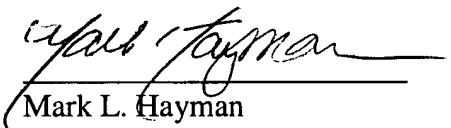
Therefore, Applicants assert that the cited prior art does not motivate one skilled in the art to perform the method of claim 3 and 5, and in addition, the present method provides results that are unexpected over the teachings of the prior art. Accordingly, Applicants assert that claims 3 and 5 are not obvious and Applicants respectfully request withdrawal of the 103(a) rejections.

III. Conclusion

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,



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